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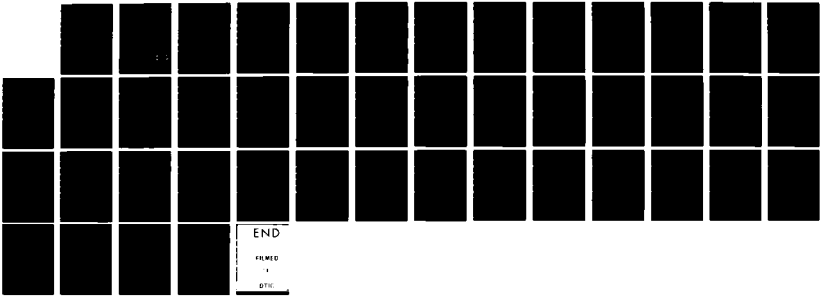

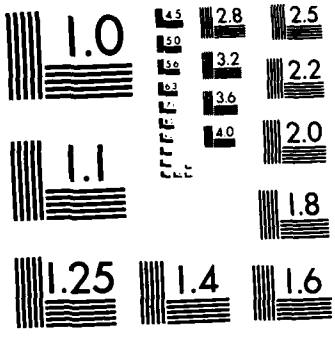

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OVIPOSITION-MODIFYING SUBSTANCES FOR MOSQUITOES

Final Report

Yih-Shen Hwang

July 1, 1981

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD-17-79-C-9026

Department of Entomology, University of California
Riverside, CA 92521

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A125421	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* OVIPOSITION-MODIFYING SUBSTANCES FOR MOSQUITOES		5. TYPE OF REPORT & PERIOD COVERED Final Report April 1979 to March 1981
		6. PERFORMING ORG. REPORT NUMBER 3
7. AUTHOR(s) Yih-Shen Hwang		8. CONTRACT OR GRANT NUMBER(s) DAMD-17-79-C-9026
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Entomology University of California Riverside, CA 92521		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62770A.3M163770A802.00.107
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research & Development Command Ft. Detrick Frederick, MD 21701		12. REPORT DATE July 1981
		13. NUMBER OF PAGES 44
14. MONITORING AGENCY NAME & ADDRESS*(if different from Controlling Office)*		15. SECURITY CLASS. *(of this report)* Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

MOSQUITOES/OVIPOSITION-ATTRACTANTS/OVIPOSITION-REPELLENTS/OVIPOSITION-MODIFYING-SUBSTANCES/CARBOXYLIC-ACIDS/OCTANOIC-ACID/NONANOIC-ACID/ORGANIC-INFUSIONS/Culex-quinquefasciatus/Culex-tarsalis/Culex-peus/Aedes-aegypti

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The objectives of this research project are to study the chemistry and the biology of oviposition-modifying substances for mosquitoes, to investigate the possibility of applying them in the management of mosquito populations, and to evaluate the role of oviposition attractants in sampling populations of female mosquitoes and ovipopulations.

To prepare for the field evaluation of the previously identified oviposition repellents, their concentration-activity relationship and species specificity

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were studied. The magnitude of repellency of the repellents was found to be directly proportional to their concentrations. Butyric acid, the major repellent component, was repellent to Cx. p. quinquefasciatus, Cx. tarsalis, Ae. aegypti, and An. quadrimaculatus at various concentrations. The acid was repellent to Cs. incidens at higher concentrations but attractive at lower concentrations.

The study on the structure-activity relationship of homologous, straight-chain aliphatic carboxylic acids as oviposition repellents revealed that octanoic, nonanoic, and decanoic acids were the most active against gravid females of Culex quinquefasciatus, Cx. tarsalis, and Aedes aegypti. Other types of compounds, such as skatole, 1-hexadecanol, and 2-methylnonanoic acid, were also repellent against Cx. quinquefasciatus.

The semi-field evaluation of oviposition repellents in experiment field ponds showed that nonanoic acid was able to repel ovipositing females for about 8 days at the 150 ppm, 4 days at 75 ppm, 2 days at 50 ppm, and 1 day at 25 ppm.

Chicken manure infusions, which had been shown to possess ovipositional attractancy in laboratory olfactometers, attracted Cx. tarsalis and Culiseta inornata for oviposition under field conditions.

In the isolation and identification of oviposition attractants from the chicken manure infusions, the infusions were steam-distilled to give an attractive distillate which, upon extraction with ether, yielded an active ether extract. Fractionation of the extract produced a repellent basic fraction, an inactive acidic fraction, and an attractive neutral fraction. Purification of the neutral fraction with column chromatography on silica gel gave a major component which showed ovipositional attractancy. Tlc and glc methods were used to determine the purity and the composition of these fractions. Spectrometric techniques, such as ir, nmr, and ms, were employed to analyze this major component of the neutral fraction.

SUMMARY

The objectives of this research project are to investigate the chemistry and biology of oviposition-modifying substances for various species of mosquitoes, to study the possibility of applying these substances in the management of mosquito populations, and to evaluate the role of oviposition attractants for sampling female adults and their ovipositional activity. We previously accomplished the isolation and identification of mosquito oviposition repellents and biological characterization of these oviposition-modifying substances. In the initial stages of the research program, our purposes are to complete preparatory work for the semi-field and field evaluation of mosquito oviposition repellents, to study the sensory physiology of oviposition repellents, and to develop procedures and techniques for the isolation and identification of oviposition attractants.

To determine the activity of the previously identified oviposition repellents quantitatively and to study the relationship between their concentrations and biological activity, we bioassayed six repellent carboxylic acids against Culex quinquefasciatus Say and Cx. tarsalis Coquillett at various concentrations in olfactometer units. The most effective repellents for Cx. quinquefasciatus were acetic and isobutyric acids, and that for Cx. tarsalis was caproic acid. Within the range of concentrations used, the magnitude of repellency of the carboxylic acids was directly proportional to the acid concentrations.

To determine the species specificity of the repellents, we bioassayed butyric acid, the major repellent component, against several species of mosquitoes other than Culex at various concentrations. Aedes aegypti L. females showed significant negative response to butyric acid at $6x10^{-1}$% concentration. Anopheles quadrimaculatus Say was significantly repelled by the acid at $6x10^{-2}$ and $6x10^{-3}$%. Culiseta incidens (Thomson) females were significantly repelled by butyric acid at $6x10^{-1}$%, but no significant response was observed at $6x10^{-2}$%. Nonetheless, at both $6x10^{-3}$ and $6x10^{-4}$%, Cs. incidens females were significantly attracted by butyric acid. Noteworthily, butyric acid showed remarkable properties of being both attractant and repellent for this species of mosquito dependent upon its concentrations.

In conducting field tests, it is very difficult to assess the effectiveness of oviposition repellents which may possess larvicidal activity. It was therefore important to evaluate these oviposition repellents for their larvicidal activity. Our preliminary studies revealed that octanoic, decanoic, and dodecanoic acids did not exert any larvicidal activity against second instars of Cx. quinquefasciatus at 1, 5, and 100 ppm concentrations.

To identify the locations of chemoreceptors which mediate negative ovipositional responses of mosquitoes, we extirpated the proboscis, all tarsal segments, and a variable number of antennal segments of Cx. quinquefasciatus females and quantitatively studied the ovipositional responses of the extirpated mosquitoes by using butyric acid as a repellent. The location of the chemoreceptors for perceiving butyric acid was thus determined to be in the antennae which could be the most important sensory organ to mediate the negative ovipositional responses.

The study on the structure-activity relationship of homologous, straight-chain, aliphatic carboxylic acids from C_5 to C_{13} as oviposition repellents

revealed that octanoic, nonanoic, and decanoic acids were, in general, the most active against gravid females of Cx. quinquefasciatus, Cx. tarsalis, and Ae. aegypti. The level of repellency was about the same among these three species of mosquitoes. The active ranges of these compounds were about 10^{-2} to 10^{-4} M. Other types of compounds, such as skatole, 1-hexadecanol, and 2-methylnonanoic acid, were also repellent against Cx. quinquefasciatus.

The semi-field evaluation of oviposition repellents was conducted by using metal-sheet cylinders in experimental field ponds. Water confined in the cylinders was first spiked with attractive chicken manure to increase mosquito oviposition and then treated with repellent octanoic acid. We found that those cylinders containing 15- or 30-ppp octanoic acid received less mosquito oviposition than the control cylinders for a period over three weeks. At a lower concentration, this carboxylic acid did not show measurable repellency under semi-field conditions.

The full-scale field evaluation of oviposition repellents was carried out in experimental field ponds. Nonanoic acid, formulated with xylene and Triton X-100, was able to repel ovipositing female mosquitoes for about 8 days at the 150 ppm, 4 days at 75 ppm, 2 days at 50 ppm, and 1 day at 25 ppm.

In the isolation and identification of oviposition attractants from the chicken manure infusions, the infusions were steam-distilled under atmospheric pressure to give an attractive distillate which, upon extraction with ether, yielded an active ether extract. Fractionation of the ether extract produced a repellent basic fraction, an inactive acidic fraction, and an attractive neutral fraction. Purification of the neutral fraction with column chromatography on silica gel gave a major component which showed ovipositional attractancy in laboratory olfactometers. Thin-layer and gas chromatographies were used to study the compositions of these fractions. Infrared, nuclear magnetic resonance, and mass spectrometric techniques were employed to determine the chemical structures of the oviposition attractants.

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ANNUAL REPORT

A. INTRODUCTION

Gravid females of many species of mosquitoes show a high degree of preference in selecting specific oviposition sites in the general area of their breeding sources. This preference may be due to the presence of oviposition pheromones or oviposition attractants and repellents in natural habitats. These oviposition-modifying chemicals regulate the ovipositional behavior of mosquitoes and thus provide a mechanism for the prevalence and occurrence of adult mosquitoes. Oviposition pheromones may occur in nature as intraspecific messengers to inform conspecifics of suitable oviposition sites. Oviposition attractants and repellents are generally believed to be produced in nature by microbial fermentation and breakdown of organic matter, and these transspecific products serve as kairomones or allomones providing cues for gravid mosquitoes to detect suitable or unsuitable oviposition sites. If these oviposition-modifying substances become known and available to us, mosquito populations can be sampled and manipulated through regulation of mosquito oviposition. Thus, these substances offer good potential as mosquito-control agents which could supplement other chemical and biological control strategies developed for vector and pest mosquitoes. Additionally, a knowledge of these behavior-modifying substances could provide a basis for understanding behavioral responses of gravid females which constitute an important portion of the total populations.

B. BACKGROUND

The presence of attractants and repellents in natural habitats for mosquito oviposition has been demonstrated by many researchers. Gerhardt (1959) reported that ovipositing female mosquitoes were attracted by stimuli, acting on their olfactory receptors and directing them to oviposition sites containing excess organic matter. In qualitative studies, Gubler (1971) showed that ovipositing females of Aedes (Stegomyia) albopictus Marks and Ae. (S.) polynesiensis Marks were repelled by high concentrations of ammonia and protein solutions, but attracted by high concentrations of organic infusions such as leaf, grass, and guinea pig chow. Gjullin et al. (1965) reported that grass infusions and log pond water increased oviposition of Ae. aegypti L. and Culex pipiens quinquefasciatus Say. Ikeshoji (1966a, 1968) showed the existence of oviposition attractants for Cx. p. fatigans Wiedemann in natural breeding water and attempted to isolate and identify them. Crude mixtures of oviposition attractants for Cx. p. quinquefasciatus, Cx. tarsalis Coquillett, Ae. nigromaculis (Ludlow), and Ae. taeniorhynchus (Wiedemann) were isolated from natural breeding water by Ikeshoji and Mulla (1970). Several workers produced evidence showing that bacteria produced certain oviposition attractants and stimulants as degradation products of organic matter (Hazard et al. 1967, Maw 1970, Ikeshoji et al. 1975).

From the evidence in the literature and from our own preliminary investigations, it is evident that, in natural breeding sites, microbial decomposition of certain organic matter produced volatile and non-volatile substances which act as oviposition-modifying factors for various mosquitoes. These substances may be species-specific or non-specific. They can be attractants, repellents, stimulants, or deterents.

In view of these successful approaches to produce mosquito oviposition-modifying substances in infusions of organic matter, we initiated exploratory studies on the biology and chemistry of some fermentation products of organic matter showing oviposition-modifying activities. After investigating infusions of various organic substrates, we found that a 1% lab chow infusion was signifivantly repellent to several species of ovipositing female mosquitoes and that a 1% chicken manure infusion was significantly attractive to Cx. p. quinquefasciatus females but repellent to Cx. tarsalis females (Kramer and Mulla (1979). These infusions were prepared by adding 1 part of lab chow or chicken manure in 99 parts of tap water and allowing the resulting suspensions to ferment at room temperature. The lab chow infusion became repellent after five days and stayed active for two more weeks. The chicken manure infusion showed attractancy from 9 to 26 days after the start of fermentation.

For carrying out a number of different chemical and biological investigations, a large amount of stock infusions was needed. It thus became necessary for us to determine the storage stability of the infusions at various temperatures. Our studies showed that both infusions remained active for ten weeks or longer when stored in a freezer at -10°C. This storage technique has enabled us to store a large quantity of active infusions for considerable periods of time. The storage stability was short-lived at 10° or 20°C.

To characterize the response of mosquitoes to the oviposition repellents, we investigated the effects of several physiological parameters on the responses of mosquitoes. We found that the interval between blood feeding and oviposition, the parous condition, and prior exposure to test infusions in bioassays did not affect the response of gravid mosquitoes to the 1% lab chow infusion.

As previously described, the infusions were not active when freshly prepared but became active as the aging process proceeded. It was suspected that bioactive compounds were produced as a result of microbial fermentation. The involvement of microorganisms in the production of attractants and repellents was determined by comparing the activity of the infusions prepared and brewed under septic and aseptic conditions. The aseptic infusions remained inactive whereas the septic infusions consistantly showed repellency or attractancy (Kramer and Mulla 1979).

The production of active compounds by microorganisms in organic infusions was thus confirmed, and the biological activity of these compounds was characterized. Consequently, chemical investigations seemed to be in order for the determination and structural elucidation of the active compounds that elicited positive or negative oviposition response in mosquitoes. In our studies on the isolation and chemical identification of the oviposition repellents, the active lab chow infusion was distilled to give an active distillate which, upon ether extraction, gave an active ether extract. Fractionation of the ether extract yielded an active acidic fraction and an inactive non-acidic fraction. Gas chromatographic analysis on Porapak R and AT-1200--H_3PO_4 columns of the acidic fraction showed the presence of acetic, propionic, isobutyric, butyric, isovaleric, and caproic acids, butyric acid constituting 85% of the total amount of these acids. In bioassay tests, these aliphatic carboxylic acids, individually and in combination, exhibited ovipositional repellency against the two species of Culex mosquitoes at the concentration of 6×10^{-2}% (Hwang et al. 1978, 1979).

C. OBJECTIVES

The objectives of this research project are:

1. To investigate the chemistry of oviposition-modifying substances for various species of mosquitoes.

The oviposition-modifying substances for mosquitoes will be isolated and purified from natural sources, and active compounds will be chemically identified or their structures will be elucidated. If the active compounds are novel and commercially unavailable, they will be synthesized. Analogues or homologues of the active compounds will also be synthesized for structure-activity studies.

2. To investigate the biology of oviposition-modifying substances.

Responses of various species of ovipositing female mosquitoes to the oviposition-modifying substances will be studied in the laboratory. The sensory physiology and the toxicity of these behavior-modifying substances will be investigated. Concentration-activity relationship and species specificity of these substances against various species of mosquitoes will be studied.

3. To study the possibility of applying oviposition-modifying substances in the management of mosquito populations.

To achieve this goal, these substances will be evaluated under semi-field and field conditions against various species of mosquitoes. This will allow us to assess the effectiveness of using the oviposition-modifying substances to manipulate ovipositional behavior of mosquitoes under field conditions.

4. To evaluate the role of oviposition attractants for sampling populations of female adult mosquitoes and ovipopulations.

The oviposition attractants will be evaluated in traps or light traps to assess their efficacy for collecting female adult mosquitoes and eggs for population and epidemiological studies. By doing so, mosquitoes that do not readily respond to conventional light traps may possibly be collected.

D. SPECIFIC AIMS

The specific aims of the present research program are:

1. To isolate and purify oviposition-modifying substances from infusions of various organic materials that possess oviposition-modifying activity by means of physical and chemical procedures in pure or semi-pure forms for chemical identification.

2. To chemically identify or to elucidate the structures of the oviposition-modifying substances with various spectrometric and chromatographic techniques.

3. To synthesize the oviposition-modifying substances, if they are novel and commercially unavailable, by means of modern methods of organic synthesis.

4. To synthesize homologues and analogues of oviposition-modifying substances for procuring more active compounds and for structure-activity relationship studies.

Physical properties and structural characteristics of these compounds will be used as parameters to correlate their chemical structures with ovipositional activity against several important species of pest and vector mosquitoes.

5. To study ovipositional responses of gravid female mosquitoes to the oviposition-modifying substances in laboratory olfactometers.

6. To investigate the sensory physiology of the oviposition-modifying substances and to identify the locations of chemoreceptors that mediate the perception of the oviposition-modifying substances in mosquitoes.

7. To evaluate the possible toxicity of the oviposition-modifying substances--the toxicity which may possibly influence the accuracy of field evaluations of the behavior-modifying substances.

8. To study the concentration-activity relationship and the species-specificity of the oviposition-modifying substances against such mosquitoes as Culex, Aedes, and Anopheles.

9. To evaluate the oviposition-modifying substances under semi-field conditions by using galvanized sheet-metal cylinders in natural mosquito-breeding ponds.

10. To evaluate the oviposition-modifying substances under field conditions by using natural mosquito-breeding ponds to assess the effectiveness of these substances in manipulating ovipositional behavior of various species of mosquitoes in the field.

11. To evaluate the feasibility of utilizing the oviposition attractants in traps or light traps for sampling populations of mosquitoes that do not readily respond to conventional mosquito light traps.

E. ACCOMPLISHMENTS

E-1. CONCENTRATION-ACTIVITY RELATIONSHIP OF OVIPOSITION REPELLENTS

Our previous studies showed that acetic, propionic, isobutyric, butyric, isovaleric, and caproic acids, individually and in combination, exhibited ovipositional repellency against Cx. quinquefasciatus and Cx. tarsalis at the concentration of 6×10^{-2}% in laboratory olfactometer units (Hwang et al. 1978, 1980). To determine the biological activity of these carboxylic acids quantitatively and to investigate the relationship between their concentrations and biological activity, we conducted the present studies (Kramer et al. 1979).

Methods and Materials. The mosquitoes used in this study were obtained from stock colonies maintained in our laboratory. Procedures used for the maintenance of laboratory colonies were described by Kramer and Mulla (1979).

In the bioassay tests, two glass stender dishes (37x25 mm with a 4.735 cm^2 surface area) were placed on a paper towel, over which a 1-liter polystyrene cup (Amoco #41032) was inverted. An aqueous solution or suspension of a test compound (4 ml) was placed in one of the dishes, and a distilled water standard (4 ml) was placed in the other. A vacuum line (flow rate, 50 ml/min) was connected to the top of the bioassay unit to provide for ventilation and to create a gradient of the volatile test compound. Five females (6-8 days post blood-feeding on chicks) were introduced into the bioassay unit between noon and 3 p.m. on the test date, and the results of oviposition were recorded the following morning. All experiments were replicated at least eight times.

The criterion for the measurement of oviposition response was the number of ovipositions in both the treatment and the standard. The activity is expressed as the oviposition activity index (OAI) and calculated as follows:

$$OAI = \frac{N_t - N_s}{N_t + N_s}$$

wherein N_t denotes the number of eggs (for Aedes) or egg rafts (for Culex) in a treated sample, and N_s denotes the number of eggs or egg rafts in the standard (untreated).

All index values determined by this formula lie within the range from +1 to -1. Positive values indicate that more ovipositions are observed in the treatment than the standard, thus evincing the compound to be attractive. Negative values indicate that more ovipositions are observed in the standard than the treatment, thus showing the compound to be repellent. The data were analyzed statistically and the significance of all indices was determined by the chi-square analysis.

All aliphatic carboxylic acids used were of reagent grade. Except caproic acid, they were dissolved in distilled water and serially diluted to the desired concentrations. Caproic acid was dissolved in acetone, and the acetone solution was serially diluted. Aliquats of the serially diluted acetone solutions were dispensed in water to make aqueous solutions or suspensions at the desired concentrations. No more than 1% of acetone was present in the resultant solutions or suspensions. In testing caproic acid in various concentrations, an equal amount of acetone was added to the standard dish.

To assess synergistic action of the six carboxylic acids, we mixed them at the original ratio found in the 1% lab chow infusion (combination 1:87.58% butyric acid, 3.79% acetic acid, 3.40% caproic acid, 3.12% isovaleric acid, 1.43% isobutyric acid, and 0.08% propionic acid) and at an equal ratio (combination 2).

Results and Discussion. Figure 1 shows the ovipositional response of Cx. quinquefasciatus and Cx. tarsalis females to the six lower aliphatic acids at the concentrations from $6x10^{-1}$ to $6x10^{-4}$%. All six acids were significantly repellent to both species of mosquitoes at $6x10^{-1}$% and to Cx. quinquefasciatus at $6x10^{-2}$%. Acetic and isobutyric acids showed significant repellency against Cx. quinquefasciatus at $6x10^{-3}$%. Significant negative responses were displayed by Cx. tarsalis to caproic acid at $6x10^{-2}$ and $6x10^{-3}$%. At lower concentrations,

these acids induced no significant responses in the mosquitoes. The concentration of the mixture of these acids in the original lab chow infusion was $6x10^{-2}$%.

Our studies revealed that the most effective repellents for Cx. quinquefasciatus were acetic and isobutyric acids and that for Cx. tarsalis was caproic acid. Within the range of concentrations used, the magnitude of repellency of the carboxylic acids was directly proportional to the acid concentrations.

To determine if there was any significant relationship between the acid concentrations used and the resulting numbers of oviposition observed, we computed the mean total number of ovipositions (treatment and standard) for each unit in the oviposition choice tests for all acids tested against both species of mosquitoes and conducted the analysis of variance. Significant F values were determined for butyric and acetic acids against Cx. quinquefasciatus and for isobutyric acid against Cx. tarsalis. Means were ranked by using Duncan's new multiple range test, and the results are shown in Table 1. These studies revealed that there was a significant inverse relationship between the acid concentrations and the mean number of total ovipositions in a unit.

Table 2 shows the ovipositional activity of combinations 1 and 2 against Culex mosquitoes. The responses of mosquitoes to both combinations were very similar at the $6x10^{-2}$ and $6x10^{-1}$% concentrations. Neither combination induced a response greater than that produced by the individual acids at the corresponding concentrations. From these investigations, it became apparent that each carboxylic acid acted individually as oviposition repellent, and no synergistic effect was found by combining these acids altogether.

E-2. SPECIES SPECIFICITY OF OVIPOSITION REPELLENTS

Because some species of mosquitoes cohabit the same aquatic environment, they might utilize the same chemical substances for selecting oviposition sites. Prior to field experiments, it was therefore essential to determine the ovipositional activity of the repellents against several species of mosquitoes other than Culex for understanding the species specificity of the repellents.

Methods and Materials. Butyric acid was found to be the major component in the repellent fraction of the lab chow infusion and was therefore used in repellency studies against several species of mosquitoes. The following species of mosquitoes were used: Culiseta incidens (Thomson), Anopheles quadrimaculatus Say, and Ae. aegypti. All three species of mosquitoes were maintained according to the procedures of Gerberg (1970).

The bioassay procedure described previously in Section E-1 was followed for An. quadrimaculatus females. However, slight modifications were made for Ae. aegypti and Cs. incidens females. For oviposition by Ae. aegypti, a 2.5x10-cm strip of paper towel (Crown No. 711) was placed in the liquid along the inside margin of all glass stender dishes to facilitate substrate oviposition of eggs by this species. In Cs. incidens test, black cloth discs (3 cm diameter) were placed under all stender dishes because a dark background had been found to induce a greater degree of oviposition in this species.

In calculating the OAI values, the number of egg rafts was used to represent

the number of oviposition in Culiseta females. In Aedes and Anopheles, the females were examined after oviposition to determine the number of females that had oviposited, and this number was correlated with the number of eggs laid in both the treatment and the standard.

Aqueous solutions of butyric acid were prepared according to the procedure previously described.

Results and Discussion. Figure 2 shows the ovipositional activity of butyric acid at various concentrations against five species of mosquitoes. Ae. aegypti females exhibited a significant negative response only at the highest concentration of $6x10^{-1}$%. An. quadrimaculatus females were significantly repelled by $6x10^{-2}$ and $6x10^{-3}$% concentrations of butyric acid. Cs. incidens females were significantly repelled by butyric acid at $6x10^{-1}$% but significantly attracted at $6x10^{-3}$ and $6x10^{-4}$%. It was noteworthy that butyric acid showed remarkable properties of being both attractant and repellent for Cs. incidens females depending upon its concentration, inducing a repellent response at higher and an attractant response at lower concentrations (Kramer et al. 1980).

From these studies, it is possible that the ovipositional behavior of various species of mosquitoes can be maneuvered by using different oviposition-modifying substances or different combinations of substances at various proportions and concentrations. It would be feasible that a particular formulation of oviposition-modifying substances could be designed for manipulating the ovipositional behavior of a given species of mosquito.

E-3. STRUCTURE-ACTIVITY RELATIONSHIP OF OVIPOSITION REPELLENTS

Butyric acid, the major constituent of the active fraction separated from the lab chow infusion, showed a distinctive species-specificity against various species of mosquitoes as reported in the previous annual report. This acid is particularly repellent to An. quadrimaculatus, moderately repellent to Cx. tarsalis and Cx. quinquefasciatus, and less repellent to Ae. aegypti and Cs. incidens.

Of the acids isolated and identified, isobutyric and acetic acids are the most repellent to Cx. quinquefasciatus and caproic acid is most active against Cx. tarsalis. Esters of these acids, such as ethyl acetate, methyl propionate, ethyl propionate, methyl butyrate, and ethyl butyrate, did not exhibit any ovipositional activity against Cx. quinquefasciatus in laboratory olfactometers.

The oviposition repellents are therefore proven to be species specific. They also display different levels of repellency with the changes in their chemical structures. In order to expand this aspect of research and to procure more active compounds, we have carried out the present study. A number of homologous, aliphatic, straight-chain carboxylic acids from C_5 to C_{13} and other compounds or materials were evaluated for their ovipositional repellency against Cx. quinquefasciatus, Cx. tarsalis, and Ae. aegypti.

Methods and Materials. All carboxylic acids, chemical compounds, and materials used in this study were obtained from commercial sources. 3-Methylnonanoic acid was synthesized in this laboratory. An acetone solution of a compound or material was dropped on a disc of filter paper (0.7-mm diameter, Whatman No. 5) until the desired

quantity of the acid was impregnated in the paper disc. Acetone was allowed to evaporate, and the paper disc was placed in a Stender dish (37x25 mm with a 4.74-cm^2 surface area) containing 4-ml distilled water. The treated dish, together with a check dish containing a blank paper disc in water, was covered with an inverted 1-liter polystyrene plastic food cup (Amoco No. 41032) and subjected to bioassay test as described in section E-1. For Ae. aegypti testing, a 2.5x11-cm strip of chromatography paper (Whatman No. 1) was placed in the liquid along the inside margin of all Stender dishes to facilitate substrate oviposition of egg by this mosquito.

Results and Discussion. The ovipositional activity of straight-chain carboxylic acids from pentanoic acid (valeric acid, C_5) to tridecanoic acid (C_{13}) is presented in Table 3. When Cx. quinquefasciatus was used in the bioassay tests, all carboxylic acids from hexanoic (C_6) acid to dodecanoic acid (C_{12}) showed 100% ovipositional repellency (OAI = 1.00) at the 1×10^{-2}M concentration. Pentanoic acid showed a high degree of activity whereas tridecanoic acid was inactive at this concentration. At 1×10^{-3}M concentration, octanoic (C_8), nonanoic (C_9), decanoic (C_{10}), undecanoic (C_{11}), and dodecanoic (C_{12}) acids were significantly repellent against this mosquito species. Octanoic and decanoic acids were significantly repellent at 3.2×10^{-4}M. Nonanoic acid was the only active compound at 1×10^{-4}M. Although the OAI value of nonanoic acid at this low concentration was merely -0.35, it was statistically significant. Nonanoic acid was therefore the most repellent compound among this series of homologous carboxylic acids tested against Cx. quinquefasciatus.

Likewise, the carboxylic acids from C_6 to C_{12} caused 100% inhibition of oviposition at the 1×10^{-2}M concentration against C. tarsalis. Pentanoic acid also showed significant repellency, but tridecanoic acid showed insignificant repellency at this concentration. C. tarsalis females were repelled by heptanoic (C_7). octanoic, nonanoic, decanoic, dodecanoic, and tridecanoic acids at 1×10^{-3}M. Of these acids, octanoic, decanoic and dodecanoic acids were the most active, causing complete inhibition of oviposition in this species at this concentration. At 3.2×10^{-4}M, decanoic and dodecanoic acids still remained repellent. Nonanoic acid was the only one that was significantly active at the lowest 1×10^{-4}M concentration. Nonanoic acid was thus proven to be the most effective acid in inhibiting C. tarsalis females from ovipositing.

When tested against Ae. aegypti at 1×10^{-2}M, octanoic, nonanoic and undecanoic acids showed complete repellency; pentanoic, hexanoic and decanoic acids showed high degrees of repellency, and heptanoic showed moderate repellency. Even at this high concentration, dodecanoic and tridecanoic acids remained inactive. At 1×10^{-3}M, pentanoic, hexanoic, heptanoic, octanoic, nonanoic and decanoic acids were significantly repellent with nonanoic acid exhibiting 100% repellency. At 3.2×10^{-4}M, octanoic acid remained significantly repellent. At the 1×10^{-4}M and 1×10^{-5}M concentrations, only nonanoic acid demonstrated significant activity against Ae. aegypti. Consequently, nonanoic acid was also the most repellent among all the compounds bioassayed (Hwang et al. 1981).

The ovipositional activity of other compounds and materials is listed in Table 4. Skatole, indole, and ammonia are some of the end products of putrefaction of organic matter. Skatole showed significant repellency at all concentrations against the gravid females of Cx. quinquefasciatus. Indole exhibited repellency only at the

highest concentration whereas ammonia did not show any measurable activity at all concentrations. High concentrations of ammonia were reported to repel Ae. albopictus and Ae. polynesiensis (Gubler 1971).

Alkanols are the precursors of alkanoic acids in fermentation of organic matter. 1-Octanol (not listed) was not active at concentrations from 7.7×10^{-6} to 7.7×10^{-4}M whereas 1-hexadecanol showed ovipositional repellency at 4.1×10^{-4} and 4.1×10^{-5}M.

2-Methylnonanoic acid, a branched-chain structural isomer of decanoic acid, was as repellent as decanoic acid showing the lowest effective concentration at 10^{-3}M. In this case, the carbon-chain branching did not seem to affect the level of repellency between the isomeric decanoic acids.

E-4. SENSORY PHYSIOLOGY OF OVIPOSITION REPELLENTS

The antennae are probably the most important external sensory organs. Antennae extirpation in Ae. aegypti females caused a definite shift from a positive olfactory response to fatty acid esters to a lack of chemical orientation (Perry and Fay 1967). Extirpation studies conducted by Ikeshoji (1966b) with Cx. fatigans Wiedemann females indicated that the antennae were important in detecting substances responsible for the stimulative factor of breeding waters.

In Cs. inornata (Williston), the two labellar lobes at the tips of the proboscis bore hairs which were proven to be chemosensory in nature (Feir et al. 1961). Chemosensory hairs were identified on mosquito tarsi which were known to mediate responses to sugar and salt solutions (Frings and Hamram 1950, Slifer 1962, Salama and Ata 1972).

The present work concerns itself with the studies on the identification of the location of chemoreceptors which mediate negative oviposition responses of Cx. quinquefasciatus females to butyric acid.

Methods and Materials. Gravid Cx. quinquefasciatus females were removed from holding cages in groups of ten each and lightly anesthesized with carbon dioxide. To determine the involvement of various organs in discriminating substances that elicit negative oviposition responses, the proboscis, all tarsal segments and/or a variable number of antennal segments were extirpated with fine surgical microscissors under a dissecting binocular microscope.

The flagellar segments of the antennae were numbered from 1-13 beginning with the proximal segment. Varying numbers of segments were removed from either/or both of the natennae. When a whole antenna was extirpated, flagellar segments 3-13 were removed. When one-half of an antenna was extirpated, flagellar segments 8-13 were removed. In experiments which involved the extirpation of portions of one antenna or the whole antenna, these cuts were always made on the right antenna. In the tarsal extirpation experiments, cuts were made just dorsal of the tibial-tarsal joints. The removal of the proboscis was made as close as possible to its base.

Both untreated and CO_2 treated females (with all body parts intact) were used in control oviposition choice tests to determine the effect of CO_2 on oviposition behavior. According to Roth (1948), continuous CO_2 anesthesia for as long as 60 minutes has no effect on the feeding behavior and recovery of Ae. aegypti females. All females which had their body part removed were allowed at least 2 hours to recover before transfer to the oviposition bioassay units.

The oviposition bioassay procedures were carried out according to those described in Section E-1.

Results and Discussion. In preliminary experiments with control and without a test substance, we found that the number of egg rafts deposited by Cx. quinquefasciatus females with various body parts extirpated (antennae, proboscis, or all tarsal segments) was not significantly different from that by normal, unextirpated females. Carbon dioxide treated females also laid as many egg rafts as those untreated.

The results of our studies are listed in Table 5. Neither treatment of carbon dioxide nor extirpation of proboscis or all tarsal segments had any significant effect on the extent of negative oviposition response of the extirpated mosquitoes to $6x10^{-2}$% butyric acid. Partial or total extirpation of one or both antennae caused the extirpated mosquitoes to cease responding to $6x10^{-2}$% butyric acid. We have therefore concluded that the chemoreceptors for the perception of butyric acid is located in the antennae which is the most important sensory organ in mediating the negative ovipositional responses of the mosquitoes to the repellents.

When exposed to $6x10^{-1}$% butyric acid (Table 6), the female mosquitoes whose various sensory organs were extirpated were still capable of making significant negative responses to this higher concentration of butyric acid. Even removal of multiple receptor system including tarsi and both antennae did not prevent the mosquitoes from responding to the concentrated acid. The only conclusion which can be deduced from the studies is that, in addition to the antennal chemorecptors, probably other mechanisms exist for the female mosquitoes to perceive the repellents (Kramer 1979).

E-5. SEMI-FIELD EVALUATIONS OF OVIPOSITION REPELLENTS

Lower aliphatic carboxylic acids were found to be oviposition repellents for several species of mosquitoes in laboratory bioassay tests (Hwang et al. 1980). Further studies showed that their higher homologues, particularly C_8, C_9, and C_{10} acids, exhibited higher levels of repellency against Culex and Aedes mosquitoes. The structure-activity relationship and the effective concentrations of these higher acids were investigated and are reported in the preceding section. To understand the working mechanism of the repellents and to explore the practicality of using them in the field, we conducted a series of exploratory semi-field experiments. The data and information obtained in the semi-field studies would provide a firm basis for conducting further full-scale field studies.

Methods and Materials. As reported in the preceding section and described in Table 3, octanoic, nonanoic, and decanoic acids are generally the most effective repellents. In addition, they are not as odoriferous as their lower homologues. In this regard, one of these acids seemed to be the compound of choice for the semi-field evaluation.

The evaluation was conducted at the Aquatic Research Facility at the University of California, Riverside. Four rectangular field ponds, 3.6 x 7.2 m each, were used in this study. Four metal-sheet cylinders, each with 46-cm height and 43- to 47-cm diameter, were tightly secured at the bottom of each pond. Each cylinder was filled with pond water. The top edges of the cylinders were a few centimeters higher than

the water level in the ponds so that water in the cylinders would be isolated from the rest of the ponds.

Two experiments were carried out. In the first experiment, the cylinders were grouped into four treatments.

Treatment 1. Containing chicken manure (0.2%) and octanoic acid (caprylic acid, 6 ppm).

Treatment 2. Containing chicken manure (0.2%) and octanoic acid (30 ppm).

Treatment 3. Containing only chicken manure (0.2%).

Treatment 4. Check (blank).

In the second experiment, the procedures in the first experiment were followed except that Treatment 1 contained 0.2% of chicken manure and 15 ppm of octanoic acid.

Previously, we reported that a chicken manure infusion was attractive to gravid female mosquitoes in the laboratory (Kramer and Mulla 1979) and in the field (see Section E-7). In evaluating oviposition repellents, we assumed that mosquito oviposition would be rather sparse. Therefore, it would be difficult to evaluate the repellent accurately. To amend this situation, we decided to spike the cylinders with chicken manure which would facilitate greater mosquito oviposition in the cylinders.

Each pond therefore contained four cylinders with four different treatments. The cylinders were placed in randomly arranged fashions to prevent positional effects. The cylinders were first treated with chicken manure one day after flooding the ponds, and, three days after flooding, octanoic acid was added into the cylinders in the first experiment. In the second experiment, chicken manure was added one day after flooding, and octanoic acid was added five days after flooding. After flooding, egg rafts were collected from the cylinders every day for 28 days in the first experiment and for 22 days in the second experiment. The egg rafts were brought to the laboratory for counting and reared until adult emergence for species identification.

Results and Discussion. Results of the first experiment are illustrated in Figure 3, in which the cumulative mean number of egg rafts per cylinder in each treatment is plotted against the number of days after flooding the ponds. Twenty-five days after the addition of octanoic acid, the cumulative mean number of egg rafts per cylinder in Treatment 2 cylinders (0.2% chicken manure and 30 ppm octanoic acid) was only about 33, whereas that in Treatment 4 cylinders (check) was 87. In Treatment 3 cylinders (0.2% chicken manure only), a total of 144 egg rafts was found. In Treatment 1 cylinders (0.2% chicken manure and 6 ppm octanoic acid), there were as many as 219 egg rafts.

From these results, we concluded that the chicken manure infusions, at the 0.2% concentration, increased the ovipositional activity of mosquitoes in the cylinders whereas octanoic acid, at the 30 ppm concentration, suppressed the ovipositional activity of mosquitoes. At the lower 6 ppm concentration, octanoic acid was not sufficient to cancel the attractiveness of the chicken manure and thus failed to suppress the female mosquitoes from ovipositing.

Figure 4 illustrates the results obtained in the second experiment. Seventeen days after the addition of octanoic acid, the accumulative mean number of egg rafts in Treatment 1 cylinders (0.2% chicken manure and 15 ppm octanoic acid) was only 34, and the number in Treatment 2 cylinders (0.2% chicken manure and 30 ppm octanoic acid) was 75. In Treatment 4 cylinders (check), an average of 111 egg rafts was found. In Treatment 3 cylinders (0.2% chicken manure), an average of 165 egg rafts was deposited. The findings not only reconfirmed the previous conclusions but also provided new information that octanoic acid was capable of suppressing mosquito oviposition at the 15 ppm concentration.

Mosquitoes collected from the cylinders were mainly Cx. tarsalis and Cx. peus Speiser.

As a result of our studies, we have been able to extend our knowledge obtained in the laboratory to the semi-field situations. We have thus proven that the aliphatic carboxylic acids manifest ovipositional repellency against gravid mosquitoes under laboratory as well as under semi-field conditions (Schultz et al. 1981).

E-6. FIELD EVALUATION OF OVIPOSITION REPELLENTS

The oviposition repellents of mosquitoes developed in this laboratory demonstrated their effectiveness both in laboratory olfactometers and in olfactory cylinders under semi-field conditions. The laboratory evaluation of the oviposition repellents was carried out under controlled conditions with confined spaces in the olfactometer, regulated air flow, and limited areas on which the repellents were applied and mosquitoes oviposited. Although natural factors, such as weather, greatly affected the outcome of the semi-field evaluation, the oviposition repellents were again confined in limited areas of water in the cylinders with rather uniform distribution of the repellents on the cylinder surfaces. The full-scale field studies would be more difficult to achieve because of numerous uncontrollable factors involved. In employing the oviposition repellents in vast areas of natural habitats of mosquitoes, dosage, evaporation rate, and distribution of the compounds must be taken into account. Here, we report our findings in this study.

Methods and Materials. Two field experiments were conducted, each with nonanoic acid as the ovipositional repellent rather than octanoic acid. Both acids showed a similar level of repellency against C. tarsalis in laboratory olfactometers (Hwang et al. 1981). The field experiments were conducted at the Aquatic Research Facility on this campus. In each experiment, nonanoic acid was formulated with a solvent and a surfactant. A surface active agent was used for obtaining relatively even distribution of the repellent on the water surface. The repellent was applied to the ponds with a 1-gal hand sprayer. During the next several days, the ponds were examined for egg rafts which were counted but not collected. Three days after applying the repellent, larval counts were also made by taking 5 dips from predetermined locations around the perimeter of the ponds. The samples were concentrated and returned to the laboratory for counting and species determination. Egg raft and larval counts were taken several times each week for several weeks.

Experiment 1.--Twelve ponds were used with 4 groups of 3 ponds each. One day after flooding, the 1st and 2nd groups were treated with 150 and 75 ppm nonanoic acid, respectively. The repellent was formulated in the ratio of 80 volume parts of nonanoic acid, 17 volume parts of xylene and 3 volume parts of Poly-Tergent G-300.

The 3rd group was treated with only the xylene and Poly-Tergent G-300 at the concentration used in the 150 ppm ponds. The 4th group of ponds remained untreated as checks.

Experiment 2.--Nine ponds were used and were placed randomly into 3 groups of 3 ponds each. On the same day of flooding, the 1st and 2nd groups were treated with 50 and 25 ppm nonanoic acid, respectively. They were formulated in a ratio of 80 volume parts of nonanoic acid, 17 volume parts of xylene and 3 volume parts of Triton X-100. The 3rd group of ponds was treated with only the xylene and Triton X-100 at the same concentration used in the 50 ppm ponds.

Results and Discussion. Prior to the 2 described field tests with nonanoic acid, preliminary field tests were conducted with octanoic acid. The acid was tested in 3.6 x 7.2-m ponds at 10, 20, 25, and 50 ppm. Although some reduction in egg-laying was found at the higher concentration, it was not significant. The reasons for this are probably twofold. First, the repellent was not evenly distributed over the surface. For this reason, a surfactant was added on all additional field tests. Secondly, some of the repellent was probably absorbed into the edges of the ponds which could not occur in the metal cylinders in the semi-field tests described above. Because of this, subsequent tests were conducted with higher concentrations.

Experiment 1.--The results of this experiment are presented in Figure 5. In the ponds treated with 150 ppm of nonanoic acid, egg rafts were not detected until 8 days post-treatment, and at 75 ppm not until 6 days post-treatment. The numbers of egg rafts in the treated ponds remained lower than those in the check ponds until 13 days post-treatment. The larval and pupal populations in the treated ponds remained lower than those in the check ponds for the entire 13 days after treatment. Although a reduction in oviposition due to the solvent and surfactant compared to the check was noted, this difference was usually not significant. The surfactant, Triton X-100, worked well in obtaining an even distribution of repellent on the water surface. At 150 ppm, the nonanoic acid could be seen on the surface as a thin film which remained about 7 days.

Experiment 2.--Since 150 and 75 ppm of nonanoic acid were proved effective in preventing oviposition in the field, we wanted to determine how effective lower concentrations would be. The result of 50 and 25 ppm of the same repellent are presented in Figure 6. Complete oviposition repellency was established until the 3rd day at 50 ppm and until the 2nd day at 25 ppm. The number of egg rafts on the treated ponds, compared to the solvent check ponds, remained lower throughout the 14 days of the experiment (Schultz et al. 1981).

E-7. FIELD EVALUATION OF ATTRACTIVE ORGANIC INFUSIONS

Previously, we reported that a 1% chicken manure infusion, fermented for 9 to 26 days, was significantly attractive to the gravid females of Cx. quinquefasciatus in laboratory bioassay tests (Kramer and Mulla 1979). For ascertaining the effectiveness of this infusion under field conditions, the present study was undertaken.

Methods and Materials. The field evaluation of the chicken manure infusion was conducted in the natural field ponds at the Aquatic and Vector Control Research Facility in Oasis, California. Eight ponds were used for this purpose. Each pond

had a measurement of 4.8 x 4.8 m and held 10,000 liters of water. Four ponds, randomly selected, were treated with chicken manure at the 1% concentration, and the other four ponds were kept untreated as control. Samples were taken every 3-4 days and brought back to the laboratory for counting and for classifying the immature mosquitoes in the samples.

Results and Discussion. The mean numbers of immature mosquitoes collected from both treated and control ponds are listed in Table 7. Each mean from the treated ponds was invariably higher than that from the control ponds. The chicken manure infusion was therefore effective in attracting female mosquitoes for oviposition. The mosquitoes collected from these ponds were predominantly Cx. tarsalis with a small number of Culiseta inornata (Williston).

E-8. ISOLATION AND IDENTIFICATION OF OVIPOSITION ATTRACTANTS

Concurrent with the oviposition repellent studies, techniques and procedures are being developed for the isolation and identification of the mosquito oviposition attractants produced in the chicken manure infusions. In the initial stages, efforts are being made to separate active fractions from inactive materials and to isolate the oviposition attractants in semi-pure or pure forms for chemical identification.

Methods and Materials. The chicken manure infusion (120 liters), fermented for nine days, was subjected to steam distillation to yield a distallate (240 liters). The distillate, 1 liter at a time, was extracted with ether (1 x 300 ml, 2 x 200 ml), and the combined extracts were dried over Na_2SO_4 and evaporated to dryness. The residual ether extract was subjected to bioassay tests.

The ether extract (2.4 g) was again dissolved in ether (100 ml) and the ether solution was extracted with 5% aqueous NaOH (3 x 30 ml). The aqueous layers were combined, washed with ether (1 x 30 ml), and acidified with conc. HCl to pH 3-4. The acidified solution was extracted with ether (3 x 50 ml). The combined ether extracts were washed with water (1 x 10 ml) and dried over Na_2SO_4. Evaporation of the ether solution resulted in obtaining an acidic fraction (0.07 g).

The ether solution, after the separation of the acidic fraction, was then extracted with 5% HCl (3 x 30 ml). The ether layer was washed with water, dried over Na_2SO_4, and evaporated to give a neutral fraction (1.27 g). The aqueous layers were combined, washed with ether (1 x 30 ml), and basified with 5% aqueous NaOH to pH 9-10. The basified solution was extracted with ether (3 x 50 ml). The ether solutions were combined, washed with water (1 x 10 ml), dried over Na_2SO_4, and evaporated to give a basic fraction (0.08 g). All these three fractions were bioassayed.

The neutral fraction was analyzed on a silica-gel, thin-layer plate developed two-dimensionally first with heptane-benzene (1:1) mixture and then with cyclohexane. This fraction was also analyzed with a gas chromatograph with a 10% Apiezon L on Chromosorb W column.

The neutral fraction (1.04 g) was chromatographed on silica gel (60 g) and eluted successively with solvents with increasing polarity. The solvent system used in this procedure was in the order of petroleum ether, 50% petroleum ether and 50% cyclohexane, cyclohexane, 50% cyclohexane and 50% benzene, benzene, 50% benzene and 50% dichloromethane, and dichloromethane (300 ml of each solvent or mixture of

solvents). The eluate was collected in 50-ml aliquot. A total of 43 fractions was collected. The composition of each fraction was monitored by gas chromatograph with an Alltech CS-10 column, an SE-30 column, and an Apiezon L column. Fractions with similar compositions were combined into 11 fractions (from Fraction A through Fraction K). Each fraction was subjected to bioassay tests.

Infrared spectra of each fraction were taken with a Perkin-Elmer model 727 infrared spectrophotometer. Nuclear magnetic resonance spectra were taken with a Varian model EM-390 90 MHz nmr spectrometer.

Two gas chromatography-mass spectrometer (GC-MS) systems were used to analyze the fraction thus obtained. The first GC-MS system consisted of a Varian Aerograph model 1500 gas chromatograph interfaced with a Finnigan model 3100 mass spectrometer. An Alltech CS-10 column was used in the gas chromatograph. The second GC-MS system used was a Varian Aerograph model 1400 gas chromatograph (with a CS-10 column) interfaced with a Finnigan model 1015 mass spectrometer attached to a System 150 data acquisition and reduction system.

During the isolation procedure, the bioassay method described in Section E-1 was used to monitor the activity of each fraction.

Results and Discussion. The chicken manure infusion, upon steam distillation, yielded an attractive distillate which was extracted with ether to give an attractive ether extract. The ether extract was fractionated to give a neutral, an acidic, and a basic fraction. The ovipositional activity of these three fractions is listed in Table 8. The basic fraction showed significant oviposition repellency against Cx. quinquefasciatus at the 2×10^{-4}% concentration whereas the acidic fraction did not exhibit any activity within the concentrations tested. The neutral fraction showed attractancy at the 2×10^{-4} and 2×10^{-3}% concentrations. It was apparent that the neutral fraction contained the oviposition attractants.

The active neutral fraction showed six spots on a silica-gel, thin-layer plate. On gas chromatographic analysis on an Apiezon column, this fraction gave five minor peaks and a major peak.

Upon column chromatography on silica gel, the neutral fraction gave a major component eluted by a cyclohexane-benzene mixture (1:1). The major component consisted of fractions designated as Fractions E, F, G, and H, which showed only one major peak in gas chromatographic analysis. This major component gave nmr signals at δ0.94, 1.25, 3.45, 4.12, and 7.57 ppm and maximum ir absorption at 1735, 1605, 1583, 1470, 1395, 1290, 1130, 1080, and 755 cm^{-1}.

Fraction A, presumably a mixture of hydrocarbons, did not show any activity in laboratory bioassay tests (Table 8). Fractions E through H constituted the major component in the neutral fraction. Fraction E displayed oviposition attractancy at 7.25×10^{-3}%, Fraction F at 4.5×10^{-3}%, and Fraction H at 1×10^{-3} and 1×10^{-2}%. At 4.5×10^{-5}%, Fraction F showed significant repellency. Fraction G was not active within the concentrations used for bioassays.

The major component (Fractions E through H) gave a mass spectrum with major peak at m/e 279, 167, 150, 149 (base peak), 113, and 104 in the first GC-MS system. In the second GC-MS system, mass spectra were scanned every 20 seconds. The total ion current was plotted against the spectrum number to give a reconstructed gas

chromatogram which showed the presence of a minor peak and a major peak. The minor peak showed m/e at 149, 147, 129, 113, 112, 111, 97, 91, 87, 84, 83, 82, 81, 73, 71, 70, 69, 67, 57, 56, 55, 44, 43, 42, 41, 40, 39, 32, 29, 28 (base peak), and 18. The major peak gave m/e at 279, 168, 167, 150, 149 (base peak), 113, 104, 83, 76, 71, 70, 69, 57, 56, 55, 44, 43, 41, 29, 28, and 27.

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Table 1. Relationship between acid concentration and the number of ovipositions (treatment plus standard) observed[a/]

Acid Concentration	Mean no. ovipositions/5 females[bc/] butyric *C. p. quinquefasciatus*	acetic *C. p. quinquefasciatus*	isobutyric *C. tarsalis*
6×10^{-6}	3.62 a	———	———
6×10^{-5}	3.37 ab	———	———
6×10^{-4}	3.12 abc	4.12 a	4.37 a
6×10^{-3}	2.62 bcd	4.00 a	3.87 ab
6×10^{-2}	2.37 cd	4.00 a	3.62 ab
6×10^{-1}	1.75 d	2.62 b	3.00 b

[a/] Results of tests with all other carboxylic acids tested against *C. p. quinquefasciatus* and *C. tarsalis* were found to be insignificant.

[b/] All values are means of 8 replicates.

[c/] Means followed by the same letter are not significantly different from one another at the 0.05 level of probability based on Duncan's multiple range test.

Table 2. Oviposition activity indices of combinations of lower carboxylic acids tested against gravid Culex mosquitoes (distilled water as a standard).

Total conc. of acids (%)	Test species and OAI (± S.E.)[a/] C. p. quinquefasciatus	C. tarsalis
	Combination 1[b/]	
6×10^{-4}	-.13±.11	+.15±.05
6×10^{-3}	-.33±.47	-.30±.44
6×10^{-2}	-.75±.25**	-.80±.12**
6×10^{-1}	- 1 ± 0 **	- 1 ± 0 **
	Combination 2[c/]	
6×10^{-4}	-.23±.20	-.11±.15
6×10^{-3}	-.31±.21	-.53±.15**
6×10^{-2}	- 1 ± 0 **	-.83±.17**
6×10^{-1}	- 1 ± 0 **	- 1 ± 0 **

[a/] ** Significant from standard at $p < .01$.

[b/] The combination consisted of 3.79% acetic acid, 0.08% propionic acid, 1.43% isobutyric acid, 87.58% butyric acid, 3.12% isovaleric acid, and 3.40% caproic acid. All values are means of 4 replicates (5 ♀♀/replicate).

[c/] The combination consisted of equal proportions of acetic, propionic, isobutyric, butyric, isovaleric and caproic acids. All values are means of 8 replicates (5 ♀♀/ replicate).

Table 3.--Oviposition activity indices (OAI) of aliphatic carboxylic acids against Culex and Aedes mosquitoes.

Acid	Conc. M	Conc. ppm	OAI[a/, b/] C. quinquefasciatus	C. tarsalis	A. aegypti
C_5	1×10^{-2}	1020.0	-0.93**	-0.90**	-0.96**
	1×10^{-3}	102.0	+0.03	-0.24	-0.29**
	1×10^{-4}	10.2	+0.04	-0.03	-0.19
C_6	1×10^{-2}	1160.0	-1.00**	-1.00**	-0.98**
	1×10^{-3}	116.0	-0.08	-0.23	-0.47**
	1×10^{-4}	11.6	0	-0.09	+0.02
C_7	1×10^{-2}	1300.0	-1.00**	-1.00**	-0.61**
	1×10^{-3}	130.0	0	-0.75**	-0.36*
	1×10^{-4}	13.0	+0.07	-0.11	-0.19
C_8	1×10^{-2}	1440.0	-1.00**	-1.00**	-1.00**
	1×10^{-3}	144.0	-0.76**	-1.00**	-0.44**
	3.2×10^{-4}	46.1	-0.85**	-0.50	-0.81**
	1×10^{-4}	14.4	-0.06	-0.40	-0.21
C_9	1×10^{-2}	1580.0	-1.00**	-1.00**	-1.00**
	1×10^{-3}	158.0	-0.89**	-0.90**	-1.00**
	1×10^{-4}	15.8	-0.35*	-0.82**	-0.81**
	1×10^{-5}	1.6	0	-0.02	-0.37*

Table 3. (continued)

Acid	Conc. M	Conc. ppm	OAI[a/, b/] *C. quinquefasciatus*	OAI *C. tarsalis*	OAI *A. aegypti*
C_{10}	1×10^{-2}	1720.0	-1.00**	-1.00**	-0.99**
	1×10^{-3}	172.0	-0.50*	-1.00**	-0.44*
	3.2×10^{-4}	55.0	-0.67*	-0.89**	-0.20
	1×10^{-4}	17.2	-0.12	-0.23	+0.12
C_{11}	1×10^{-2}	1860.0	-1.00**	-1.00*	-1.00**
	1×10^{-3}	186.0	-0.72**	-0.45	+0.12
	1×10^{-4}	18.6	-0.02	0	+0.25
C_{12}	1×10^{-2}	2000.0	-1.00**	-1.00**	-0.29
	1×10^{-3}	200.0	-1.00**	-1.00**	+0.05
	3.2×10^{-4}	64.0	-0.45	-0.73**	-0.18
	1×10^{-4}	20.0	0.07	-0.50	-0.25
C_{13}	1×10^{-2}	2140.0	-0.13	-0.67	0
	1×10^{-3}	214.0	+0.09	-0.60*	+0.06
	1×10^{-4}	21.4	-0.08	-0.07	+0.25

[a/] Positive OAI values indicate attractancy, and negative OAI values indicate repellency.

[b/] *Significant from standard at $P < 0.05$; **significant from standard at $P < 0.01$.

Table 4. The ovipositional activity of miscellaneous compounds and materials against Cx. quinquefasciatus.

Material	Concn. (M or ppm)	OAI[a/]
Skatole	7.6×10^{-4} M	-0.94**
	7.6×10^{-5}	-0.40*
	7.6×10^{-6}	-0.40*
Indole	8.5×10^{-4} M	-0.94**
	8.5×10^{-5}	-0.22
	8.5×10^{-6}	-0.02
1-Hexadecanol	4.1×10^{-4} M	-0.89**
	4.1×10^{-5}	-0.62**
	4.1×10^{-6}	-0.05
2-Methylnonanoic acid	1×10^{-2} M	-1.00**
	1×10^{-3}	-0.71**
	1×10^{-4}	-0.16
MGK-11	100 ppm	-0.78**
	10	+0.20
	1	+0.17
Delphene	100 ppm	+0.44*
	10	+0.20
	1	-0.36
"OFF"	100 ppm	-0.85*
	10	+0.25
	1	-0.13
Dibutyl phthalate	3.6×10^{-4}	-0.33
	3.6×10^{-5}	+0.12
	3.6×10^{-6}	-0.20
NH_4OH	1×10^{-2} M	-0.18
	1×10^{-3}	+0.10
	1×10^{-4}	0
	1×10^{-5}	-0.12

[a/]**Significant at $p<0.01$. *Significant at $p<0.05$.

Table 5. Oviposition responses of C. p. quinquefasciatus females subjected to various extirpations to $6x10^{-2}$% butyric acid.

Organs extirpated	Oviposition responses[a] OAI ± S.E.[b]	$\bar{x}$ ovipositions/5 ♀♀
Intact	-.74±.10**	3.75
Intact (CO_2)	-.67±.10**	3.63
All tarsi	-.77±.11**	3.38
Proboscis	-.65±.11**	4.25
Both antennae	-.25±.15	3.13
One antenna	-.19±.17	4.37
½ both antennae	+.34±.22	4.37
½ one antenna	-.25±.27	3.00

[a] All values are means of 8 replicates.

[b] ** Significant from standard at $p < .01$.

Table 6. Oviposition responses of C. p. quinquefasciatus females subjected to various extirpations to $6x10^{-1}$% butyric acid.

Organs extirpated	Oviposition responses[a]	
	OAI ± S.E.[b]	$\bar{x}$ ovipositions/5 ♀♀
Intact	-1±0**	2.75
Intact (CO_2)	-1±0**	2.62
All tarsi	-.92±.08**	2.13
Both antennae	-1±0**	3.13
One antenna	-1±0**	2.88
All tarsi and antennae	-1±0**	1.63

[a] All values are means of 8 replicates.

[b] ** Significant from standard at $p < .01$.

Table 7. Field evaluation of 1% chicken manure infusion in Oasis, California

Days After Treatment	Mean No. of Immature Mosquitoes[a/]	
	Treated	Control
4	61	40
8	230	98
11	229	106
14	281	62
21	238	123

[a/] Means based on 4 replicates.

Table 8. Oviposition activity indices of fraction obtained from chicken manure infusion against Cx. quinquefasciatus.

Fraction	Concn (%)	OAI[a/]
Basic	$2x10^{-5}$	-0.14
	$2x10^{-4}$	-0.53**
	$2x10^{-3}$	+0.23
Acidic	$1.75x10^{-5}$	+0.29
	$1.75x10^{-4}$	0
	$1.75x10^{-3}$	-0.23
Neutral	$2x10^{-5}$	-0.14
	$2x10^{-4}$	+0.32*
	$2x10^{-3}$	+0.70**
	$2x10^{-2}$	-0.17
A	$1x10^{-5}$	+0.12
	$2x10^{-5}$	+0.19
	$4x10^{-5}$	0
E	$7.25x10^{-4}$	+0.06
	$7.25x10^{-3}$	+0.65**
	$7.25x10^{-2}$	+0.28
F	$4.5\ x10^{-5}$	-0.40*
	$4.5\ x10^{-4}$	0
	$4.5\ x10^{-3}$	+0.53**
G	$1x10^{-4}$	-0.23
	$1x10^{-3}$	+0.13
	$1x10^{-2}$	+0.27
H	$1x10^{-4}$	0
	$1x10^{-3}$	+0.50*
	$1x10^{-2}$	+0.62*

a/ *Significant at $p<0.05$

** Significant at $p<0.01$

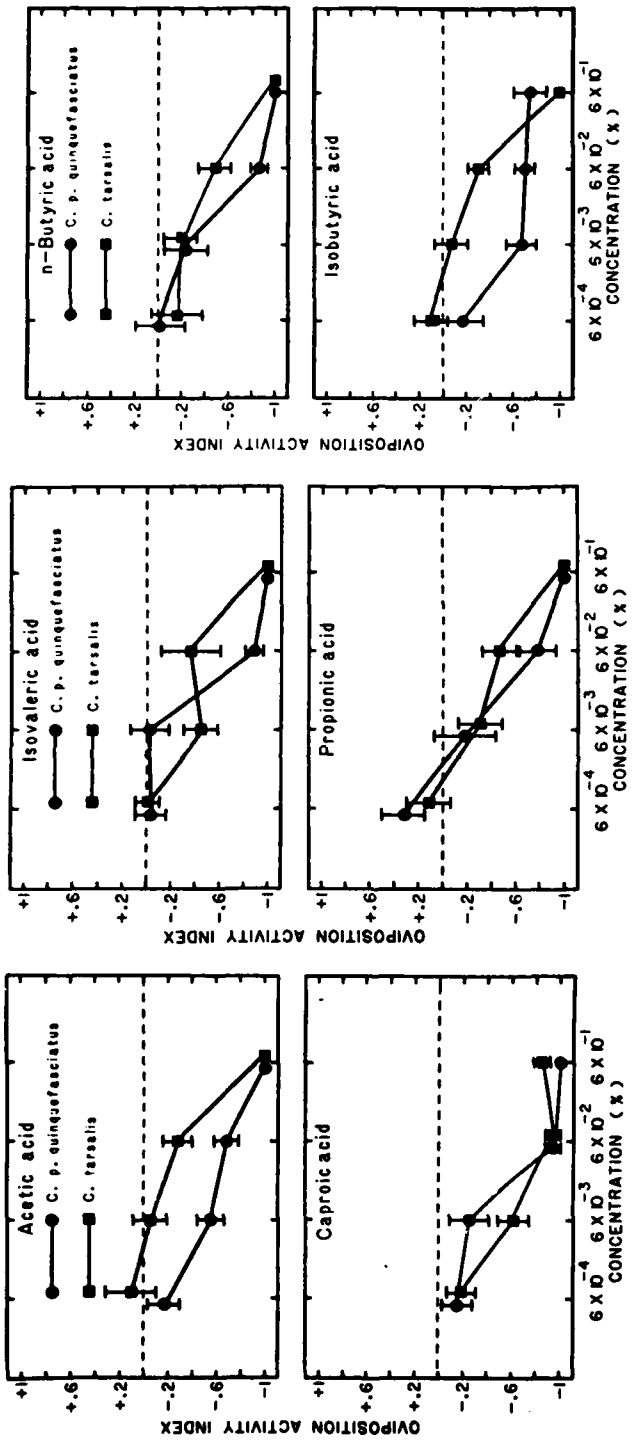

FIG. 1. Oviposition activity indices (OAI) of carboxylic acids tested against *C. p. quinquefasciatus* and *C. tarsalis* females. All values are means of eight replicates (5 ♀♀ /replicate) ± SE.

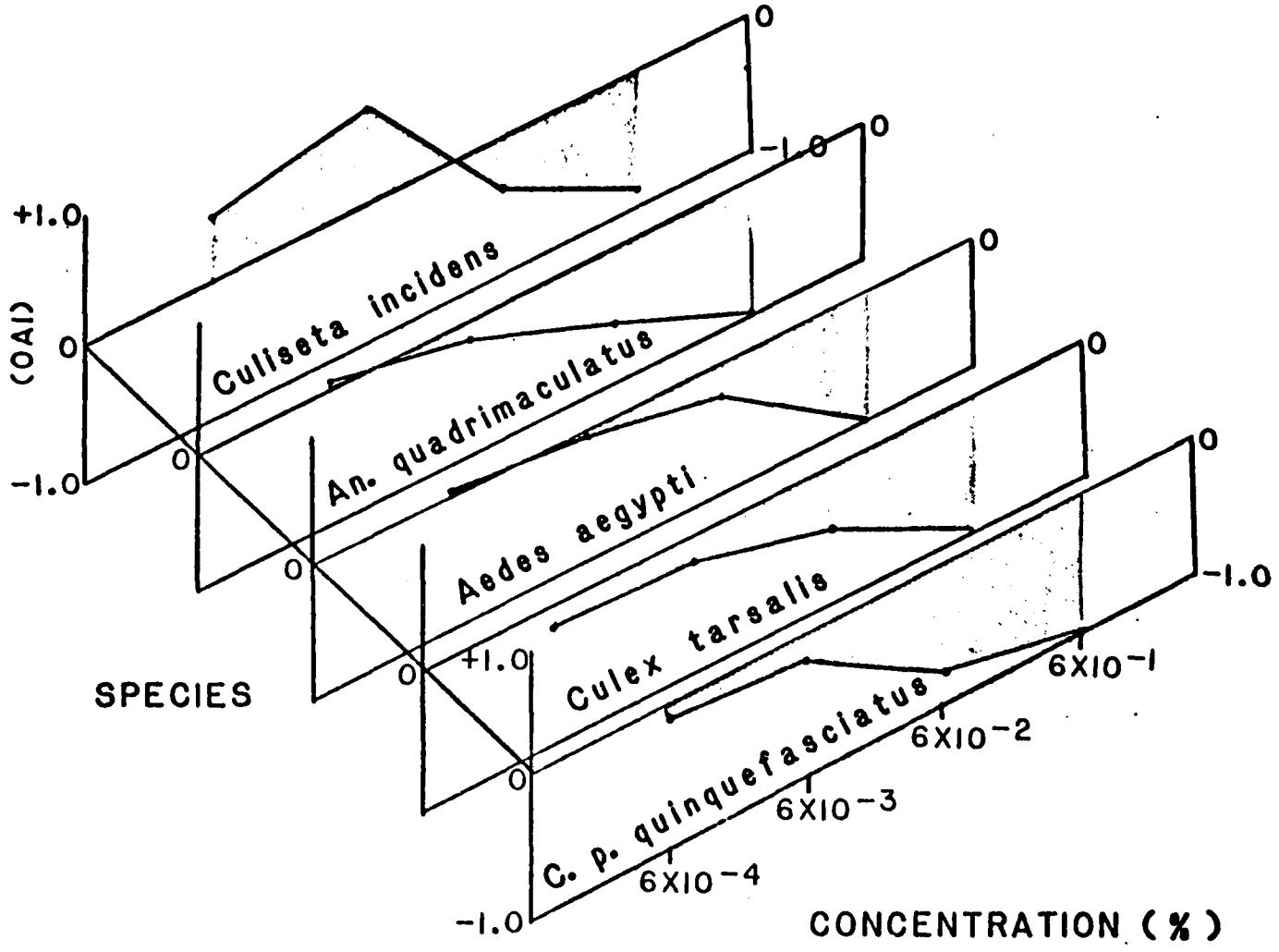

FIG. 2. OAI of butyric acid tested against different mosquito species.

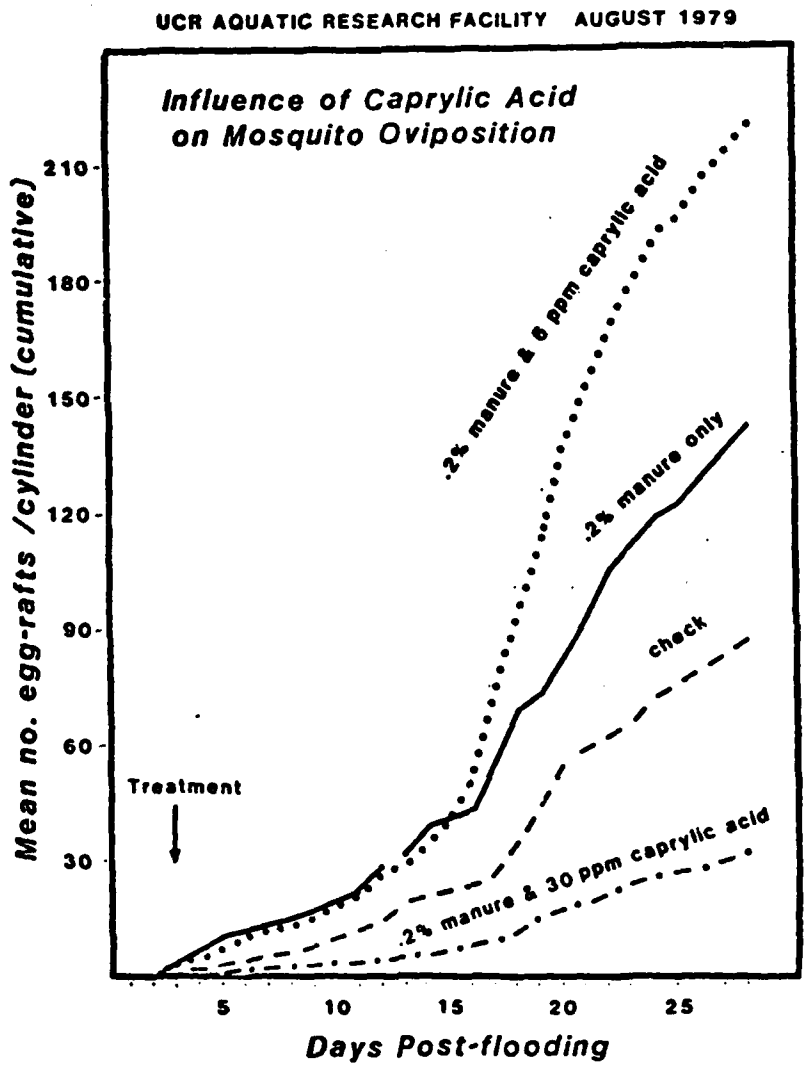

Figure 3. The ovipositional repellency of octanoic acid at 6 and 30 ppm under semi-field conditions.

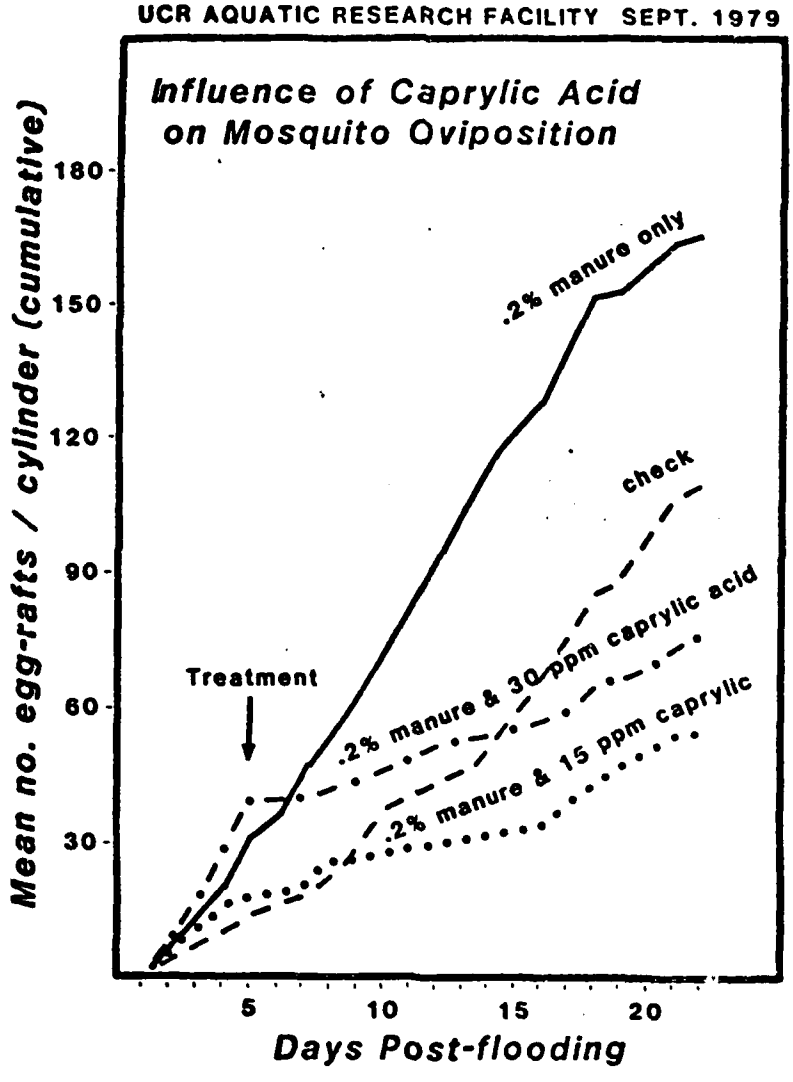

Figure 4. The ovipositional repellency of octanoic acid at 15 and 30 ppm under semi-field conditions.

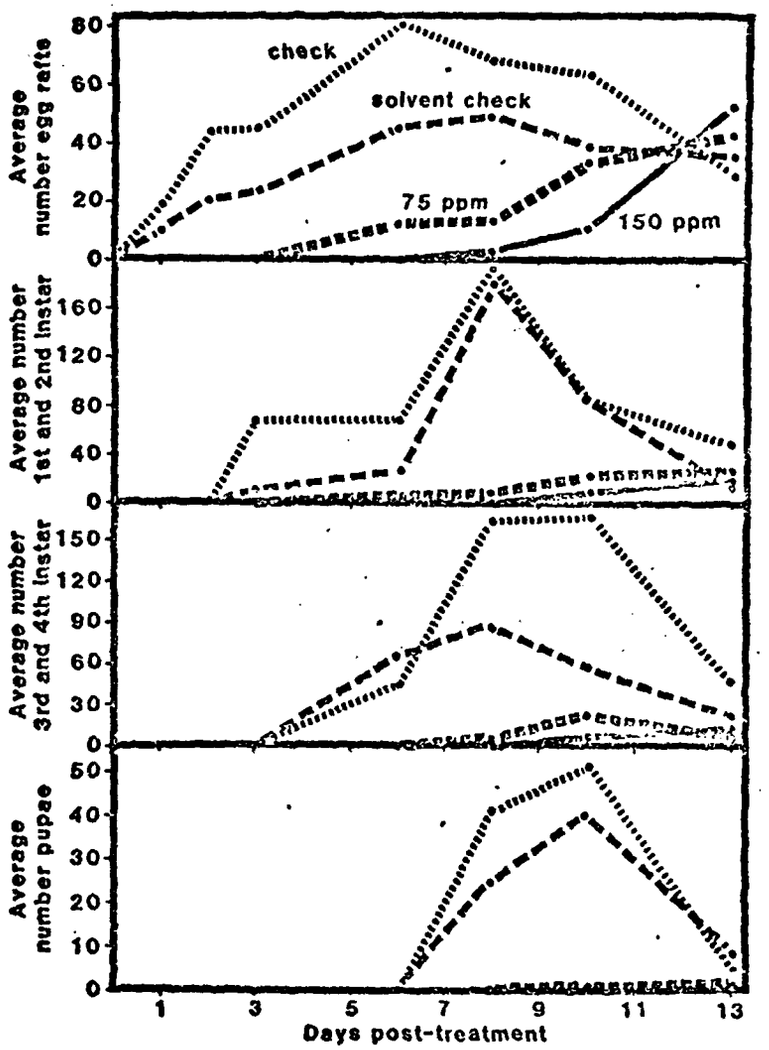

Fig. 5. Effect of nonanoic acid as the ovipositional repellent on egg rafts, larvae, and pupae in a field test (Experiment 1).

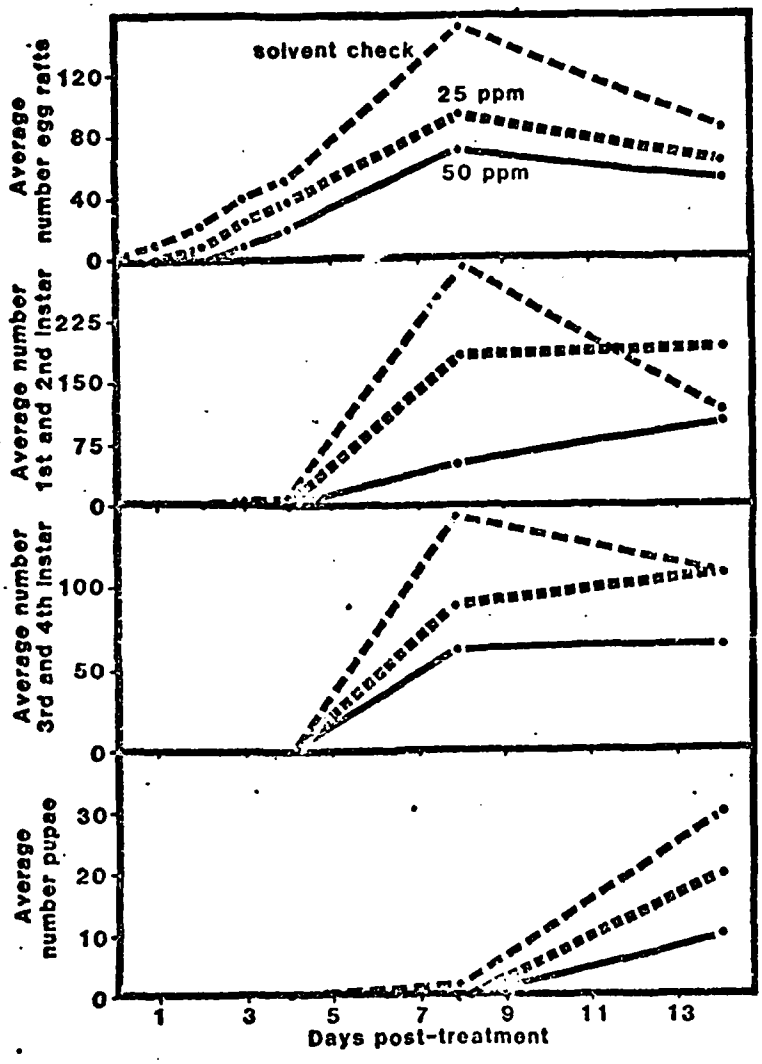

Fig. 6. Effect of nonanoic acid as the ovipositional repellent on egg rafts, larvae, and pupae in a field test (Experiment 2).

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* All publications were supported by the present contract.

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